

WHAT IS CLAIMED IS:

- Sub D'*
1. A process for producing relaxin from a non-naturally occurring prorelaxin wherein said prorelaxin comprises a leader peptide, the B-chain, a non-naturally occurring C-chain, and the A-chain, and wherein said leader peptide comprises a cleavage site adjacent the B-chain and wherein said non-naturally occurring C-chain comprises cleavage sites adjacent the B-chain and the A-chain, which method comprises removing the said leader and non-naturally occurring C-peptide from said prorelaxin using a cleaving agent at said cleavage sites and recovering relaxin.
2. The process of Claim 1 wherein the relaxin is H2 human relaxin.
- Sub B'*
3. The process of Claim 1 wherein said cleaving agent is one or more enzymes.
4. The process according to Claim 3 wherein said enzymes are selected from the group consisting of endoproteinase Asp N, trypsin, endoproteinase Lys C, endoproteinase Arg C and carboxypeptidase B.
5. The process according to Claim 4 wherein ~~said enzymes~~ are ~~trypsin or endoproteinase Arg C in combination with carboxypeptidase B~~.
- Sub D²*
6. The process according to Claim 4 wherein said enzymes are Lys C in combination with carboxypeptidase B.
7. The process according to Claim 4 wherein said enzymes are Asp N in combination with Lys C.
8. The process according to Claim 1 wherein said prorelaxin is produced recombinantly by providing DNA encoding it within an operative expression vector

transfected into host cells, culturing said transfected cells and isolating said prorelaxin.

9. The process according to Claim 8 wherein said isolation includes solubilizing and refolding said prorelaxin.

10. The process of Claim 9 wherein said prorelaxin is solubilized with a solution comprising guanidine hydrochloride.

11. The process of Claim 9 wherein said prorelaxin is refolded under conditions of dilute protein concentration.

12. The process of Claim 9 wherein prorelaxin is refolded using a redox buffer.

13. The process of Claim 8 wherein said host cells are E.coli.

14. The process of Claim 1 further comprising cyclizing the A-chain N-terminal glutamine.

15. The process of Claim 14 wherein the relaxin A-chain N-terminal glutamine is cyclized through a heat step.

D 16. The process according to Claim 1 wherein said non-naturally occurring C-peptide is comprised of the amino acid sequence KRKPTGYGSRKKR.
a (SEQ ID NO:3)

D 17. The process according to Claim 1 wherein said non-naturally occurring C-peptide is comprised of the amino acid sequence DKKRTGYGSRKKR.
a (SEQ ID NO:31)

D 18. The process according to Claim 1 wherein said non-naturally occurring C-peptide is comprised of the amino acid sequence DKKRTGYGSRKKR.
a (SEQ ID NO:32)

D 19. The process according to Claim 1 wherein said non-naturally occurring C-peptide ^{comprise} is comprised of the amino acid sequence KRKPTGYGSRRRK. (SEQ ID NO:33)

D 20. The process according to Claim 1 wherein said leader sequence ^{comprise} is comprised of the amino acid sequence MKKNIAFLLKR. (SEQ ID NO:1)

Sub
D³ 21. The process according to Claim 1 wherein said relaxin is recovered by a process comprising use of adsorption chromatography, ion-exchange chromatography, reverse-phase chromatography, size-exclusion chromatography and/or ultrafiltration.

D 22. The process according to Claim 1 optionally comprising formulating the relaxin in formulation buffer.

D 23. A prorelaxin comprising a leader peptide, the B-chain, a non-naturally occurring C-chain, and the A-chain, wherein said leader peptide comprises a cleavage site adjacent the B-chain and wherein said non-naturally occurring C-chain comprises cleavage sites adjacent the B-chain and the A-chain.

24. The prorelaxin according to Claim 23 wherein said cleavage sites are enzymatically cleaveable.

D 25. The prorelaxin according to Claim 23 wherein said non-naturally occurring C-peptide ^{comprise} is comprised of the amino acid sequence KRKPTGYGSRKKR. (SEQ ID NO:3)

D 26. The prorelaxin according to Claim 23 wherein said non-naturally occurring C-peptide ^{comprise} is comprised of the amino acid sequence DKKRTGYGSRRRK. (SEQ ID NO:31)

D 27. The prorelaxin according to Claim 23 wherein said non-naturally occurring C-peptide ^{comprise} is comprised of the amino acid sequence DKKRTGYGSRKKR. (SEQ ID NO:32)

D 28. The prorelaxin according to Claim 23 wherein said
a non-naturally occurring C-peptide ~~is comprised~~ of the
amino acid sequence KRKPTGYGSRRRK. (Seq ID 10:33)

D 29. The prorelaxin according to Claim 23 wherein said
a leader sequence ~~is comprised~~ of the amino acid sequence
MKKNIAFLLKR. (Seq ID 10:1)

30. An isolated DNA encoding the prorelaxin of Claim 23.

31. An expression vector operatively containing the DNA
according to Claim 30.

32. A host cell transfected with the vector of claim 31.

add
D4